bio-protocol Preprint

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Western blots

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An abbreviated version of this protocol was published in eLIFE in Oct 2018

OGT binds a conserved C-terminal domain of TET1 to regulate TET1 activity and function in development

DOI: 10.7554/eLife.34870

Detailed protocol

1. Prepare protein lysates in 1x SDS sample buffer
2. Boil lysates 5 min at 95C
3. Run lysates on SDS-PAGE gel in 1x Laemmli running buffer
4. Soak membrane in MeOH, then in Transfer Buffer + 10% MeOH.
5. Transfer 1 hr at 70V at room temp or overnight at 25V at 4C in 1X Transfer Buffer +10% MeOH
6. Block in PBST + 5% nonfat dry milk for 10 mins – 1 hour at room temp, or overnight at 4C
7. Incubate 1hr at room temp or overnight at 4C in primary antibody diluted in PBST
8. Wash 2 x 10 min in PBST
9. Incubate 1hr at room temp in HRP-conjugated secondary antibody diluted 1:10,000 in PBST
10. Wash 2 x 10 min in PBST
11. Drain and develop using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher) (incubate membrane with substrate for 5 minutes)
12. Expose film in dark room, develop

4x SDS Sample Buffer
4mL of 100% glycerol
2.4mL of 1M Tris pH 6.8
0.8g SDS
4mg Bromophenol blue
0.5mL BME
to 10 mL w/ H2O

10X Laemmli Running Buffer
30 g Tris Base
144 g Glycine
10 g SDS
to 1L w/ H2O

10X Transfer Buffer
30 g Tris Base
144 g Glycine
to 1L with H2O

1X Transfer Buffer +MeOH
100 mL 10X Transfer Buffer
100 mL MeOH
to 1L with H20

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hrit, J. (2019). Western blots. Bio-protocol Preprint. bio-protocol.org/prep18.
2. Hrit, J., Goodrich, L., Li, C., Wang, B., Nie, J., Cui, X., Martin, E. A., Simental, E., Fernandez, J., Liu, M. Y., Nery, J. R., Castanon, R., Kohli, R. M., Tretyakova, N., He, C., Ecker, J. R., Goll, M. and Panning, B.(2018). OGT binds a conserved C-terminal domain of TET1 to regulate TET1

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